1977

Crevicular Fluid in Children

Rafik Ferdinand Hakim
Loyola University Chicago

CREVICULAR FLUID IN CHILDREN

by

Rafik Ferdinand Hakim

A Thesis Submitted to the Faculty of the Graduate School of

Loyola University of Chicago in Partial Fulfillment of

the Requirements for the Degree of

Master of Science

June

1977

TABLE OF CONTENTS

DEDICATION

For my father, Ferdinand Alfred Hakim, and my mother, Renée Tawaf Hakim, for the sacrifices they went through to educate me and for the help they gave me in accomplishing my studies.

For my wife, Hiam Abou-Merhi Hakim, for the effort, the encouragement and the understanding she gave me which allowed me to reach my goal.

ACKNOWLEDGMENTS

My sincere gratitude and appreciation to the following:

To Wayne E. Milos, D.D.S., M.S., Director of Pedodontic Graduate Students, my teacher and main advisor, for his support and guidance in preparing this thesis.

To Patrick D. Toto, D.D.S., M.S., Director of Graduate Studies, my teacher and advisor, for his valuable ideas in the scientific research of this thesis.

To Eugene Grandel, D.D.S., M.S., Chairman, Department of Pedodontics, my teacher and advisor, for his close guidance in the design of this thesis.

I would like, also, to address my gratitude to the following companies which have loaned me the materials used to accomplish this thesis:

To Harco Electronics, Ltd., Winnipeg, Canada, for use of the Periotron.

To the Janar Company, Grand Rapids, Michigan, for the Gindex™.

VITA

Rafik Ferdinand Hakim was born in Cairo, Egypt on May 24, 1951. After graduating from "College des Frères" in 1970, he attended the French Faculty of Medicine, School of Dentistry at St. Joseph University in Beirut, Lebanon. A dental career was pursued from 1970 to 1975. In June, 1975, he received the degree of "Docteur en Chirurgie Dentaire" which is equivalent to Doctor of Dental Surgery. Since that time, Doctor Hakim has been a post-graduate student in the Department of Pedodontics, Loyola University School of Dentistry, Maywood, Illinois, and has been enrolled in the Department of Oral Biology of the Loyola Graduate School working toward a Master of Science degree in Oral Biology.

During his two years of graduate study Doctor Hakim also served as an instructor of pedodontic specialty in the Dental Assistant Program and as an instructor in the Oral Pathology Department of Loyola University School of Dentistry.

CONTENTS OF APPENDICES

APPENDIX PAGE

CHAPTER I

INTRODUCTION

Most investigations concerning periodontal disease and its relationship to gingival crevicular fluid flow have been focused on adults. Very little interest has been given to children and young adolescents. The crevicular fluid (CF) is a matter of concern for all the investigators since there is no agreement on the origin and the nature of that fluid. Most of the investigators found that the CF is an inflammatory exudate, for other researchers it is an altered serum transudate in clinically normal tissue becoming an exudate only with marked inflammation (Cimasoni 1974, Weinstein et al., 1976). There is a unanimous opinion that the CF flow is highly correlated with the severity of gingival inflammation under different clinical circumstances (Brill and Bjorn 1959, Mann 1963, Egelberg 1964, Oliver et al., 1969, Golub et al., 1971). Gingival disease in children is epidemiologic in nature (McCall 1938, Ramfjord 1968). Some indicated a varying prevalence of gingival disease in children ranging with a low of 9.1% in children 5 years old to 93% in young adolescents (Massler 1950, Greene 1960). Golub et al., Mann, Sandalli et al., (1974) demon-

strated the direct relationship between the increase of CF flow and the amount of gingival inflammation present in adults as well as in children.

Some authors suggested that the periodontal problems which occur in adults have their origin in the early years of adolescence (Baer, Ciamsoni 1974). The need to understand the relationship that exists between normal and inflammed gingivae in children is important in detection and early diagnosis. The prevention and treatment of periodontal disease in children in an early stage might reduce its occurrence in adulthood. If we can detect the primary subclinical signs of gingival inflammation, we can prevent further complications and establish early treatment. This study was conducted to learn more about CF measurements in children and the relationship of that fluid between normal and inflammed gingiva.

CHAPTER II

REVIEW OF THE LITERATURE

The specific locus of the gingival sulcus has always been of extreme interest to the periodontist. The implication of that area in the early pathogenesis of periodontal disease provoked a large amount of investigation. Individual susceptibility to periodontal disease may depend on the presence or absence of defense mechanisms in this area. The purpose of the crevicular fluid (CF) flow has been conjectured for more than fifty years. The CF was thought to play a protective function by flushing foreign bodies and bathing the sulcus with protective elements.

In the 1950's, Brill and Krasse injected fluorescein, which binds plasma proteins, into dogs. They were able to collect the fluorescein in 90% of healthy sulci, its presence on other epithelial surfaces was not observed. There are three mechanisms for the excretion of the CF: A) through the intact epithelium of the sulcus; B) through the region of the epithelial attachment; C) and through small lesions in the pocket epithelium (Brill, Bjorn and Krasse 1958, 1959, 1960). When Brill and Bjorn repeated the study on humans,

they found 96% of the sulci binding the fluorescein to the plasma protein (1959). The strips of paper they were using in the inflammed pockets were more fluorescent showing an increase in plasma proteins. The authors noted two possible mechanisms for fluid passage: 1) as a result of surrounding tissue inflammation; or 2) as a result of a normal property of the pocket epithelium which permits it to act as a semi-permeable membrane. In 1960, Brill found in another study that capillary permeability has an intimate relationship with the amount of gingival fluid obtained in the sulci. When he fed his animals with a soft diet, a highly significant relationship was observed between the clinical observation of increased gingival fluid flow and the histological findings of increased inflammation.

Krasse and Egelberg proposed that if the CF passes through intact tissues, it should contain a similar ratio of sodium and potassium as seen in the plasma (1962). They concluded that the CF cannot be considered as a simple filtration product but rather as an inflammatory exudate. The presence of inflammatory cells in the healthy gingival tissues is supporting evidence. As mentioned before, Mann (1963) proved that CF flow was directly related to pocket depth and gingival inflammation. At the same time, he found that statement correct for both adults and children. Inflammation had a higher statistical significance in relationship to the amount of fluid flow than to pocket depth. On the other hand, Salkind et al., (1963) found that

ingested sodium fluorescein did not appear in the saliva of the patients until they flushed the gingival pocket fluid through mastication. The mechanism of mastication was apparently able to pump and stimulate the gingival pocket fluid expelling it into the oral cavity.

In early literature, the CF was thought to be composed of amino acids, plasma proteins, fibrinolytic factors, cellular material, potassium, calcium and phosphorus (Weinstein 1964). More recently, the composition of the CF was more detailed and a list of the components will be presented later (Cimasoni 1974). In a study concerning the identification of protein components in the CF, a similarity of proportions among the proteins in serum and pocket fluid and the presence of fibrinogen was found (Mann and Stoffer 1964). It was that similarity which initiated the idea that CF originated as an inflammatory exudate. The chemical analysis of CF has shown the presence of several serum proteins (Loe 1965). The flow of fluid from the normal gingival crevice appeared to be slight, but increased after mechanical stimulation of the teeth or the gingiva. In the case of gingival inflammation, the rate of flow is markedly increased (Brill, Bjorn, Mann 1959). This exudate has been found to contain neutrophilic leukocytes almost always in the inflammatory reaction of the gingiva. Loe and Jensen (1965) gave important consideration to the fact that flow of fluid regularly starts before structural changes can be ascertained at the clinical level. They found also that it

persists some time after clinical inflammation has subsided. Many authors are in contradiction in their studies about what is considered normal gingiva since most often a slight subclinical inflammation exists in almost all patients considered to have healthy gingiva.

Additional studies of the gingival fluid regarding the organic and inorganic components were presented (Weinstein, Mandel, Salkind, Pappas 1967). A normal complement of organic components are present but in somewhat different concentrations than in the serum. There also seems to be major differences in the relative concentrations of inorganic components. For these reasons gingival fluid cannot be considered purely a transudate of serum. The authors explained that the mechanism of gingival fluid production is a transudate of serum modified by the cells of the sulcular area. For others the CF arises from the bloodstream, passes through the connective tissue and enters the gingival pockets through their epithelium (Brill, Bronnestam 1960).

In recent literature, Oliver et al., Golub et al., (1971) came across the same conclusion stated earlier in this paper that the CF flow increases with the severity of gingival inflammation. As the plaque accumulates upon the teeth and surrounding tissues, the appearance of clinical signs of inflammation follow, and subsequently there is an increase in the CF flow (Loe and Holm Pederson). Borden et al., (1975) found that the relationship between gingival

inflammation and CF was valid for different age groups as well as different regions of the dentition. Also in the recent literature, the presence or absence of CF in healthy gingiva is based on the method of collection. Investigators used either an intracrevicular method or the collection was made at the crevice orifice (Oliver 1969 and Golub et al., 1971). Whether the CF is considered to be an inflammatory exudate or an altered serum transudate, Kaslick et al., (1970) found that sodium was at a lower concentration than in serum. Golub et al., (1971) found that the urea was at a much higher concentration in CF than serum.

Clinical criteria for gingival health are too insensitive to detect the earliest stages of gingival disease since normal appearing tissues often exhibit, in addition to crevicular fluid, histological evidence of inflammation (Oliver 1969). Clinical visible changes in the gingival tissues should be considered as representing a secondary rather than a primary stage of inflammation. The pH of the plaque is generally alkaline, which is mainly due to the high level of plaque ammonia (Singer and Kleinberg 1975). The main source of ammonia, at least in the early stages, appears to be salivary urea (Frostell 1960). Other authors found that it is essentially the deamination of amino acids by the microorganisms (Kleinberg 1970). This suggests that ammonia may have an initiating effect on gingivitis.

In the composition analysis of the fluid, urea has been found at a high level. The urea in the crevice would replace salivary urea as a source for ammonia production.

The CF has a collagenase activity and that is the only enzyme capable of degrading native collagen. Some investigators claimed that the collagenase was produced by bacteria inhabiting the crevice (Gibbons and MacDonald 1961). On the other hand, other investigators concluded that non-collagenase producing bacteria in the flora can induce gingival inflammation (Theilade et al., 1966, Fitzgerald et al., 1960 and Jordan et al., 1965). Fullmer et al., (1970) added that the gingival inflammation can induce and stimulate the production of periodontal tissue collagenase.

Early literature was not focused on the child nor the adolescent. In the 1940's, some authors were interested in the study of periodontal disease in children (Orban 1941, Zappler 1948, Gottlieb 1944). The evaluation of CF was not a subject of research and most of the literature was concerned with the clinical observation and the relationship that existed between periodontal disease and the etiology.

The research of CF in youngsters started in the 1950's. Some researchers compared adults with children (Massler and Schour 1952, Ramfjord 1961, 1968, Mann 1963, Golub and Borden and Kleinberg 1971). They demonstrated that the CF of adults and children increased in direct ratio to the amount of gingival inflammation. Borden et al.,

(1974) examined the relationship of age and sex on urea concentration and on the pH and volume of gingival crevicular fluid and found that the CF volume increased and urea concentration decreased with inflammation. Golub and Smith (1974) found that permanent versus primary tooth type had no effect on fluid volume, crevice depth, urea concentration or crevice pH in children of the same age. Young children showed less crevicular fluid volume, shallower crevices and lower pH than the adolescents in the non-inflammed gingiva situation. Similar changes occur in children as in adults with increased inflammation. The pH, however, did not show an observable change. Turesky (1961) made a histological observation regarding early calculus formation in children and adults. He stated that the major components of the soft plaque were bacteria with a scattering of leukocytes and epithelial cells contained within an amorphous matrix. In a second phase, the calcification usually occurs. His conclusions were that under 12 years of age no calcification occurred even after 30 days of plaque accumulation. Patients older than 13 years of age start forming calculus the second day but the majority (85%) after the eighth day. Turesky found that the microbial components of non-calcified plaques are a meshwork of diffusely distributed Gram negative cocci with occasional rod forms, rarely isolated Gram positive cocci. The bacterial content changed with the degree of calcification. Gram negative filaments and threads become prominent until they

exceed the other bacteria. Also, the calculus formation is induced at a slower rate in children than in adults due mainly to the alteration of the local environment. Calculus occurs less rapidly but presents the same histologic features (Turesky 1961). In systemic qualitative and quantitative research the flora in the gingival crevice area of children was investigated (MacDonald and Aranjo 1964). The results of their investigation are summarized in Table IX (see Appendix J). The proportions of these groups of bacteria did not differ statistically from those reported in adults. However Bacteroides melaninogenicus and spirochetes were not found as commonly as in adults. The B. melaninogenicus was involved in frequent combination in the mixed anaerobic population and was shown to produce collagenolytic activity. The authors felt its infrequent occurrence and low numbers in children may be related to the low incidence of periodontal disease in children. On the other hand, spirochetes have been reported to increase in proportion to the sulcus bacterial population in periodontally involved teeth in adults. Another interesting conclusion in the study of MacDonald was to note that the gingival crevice area did not contain large numbers of streptococcus salivarius or staphylococcus.

The periodontal disease entity most frequently observed in the young patients is marginal gingivitis; a soft tissue lesion without apparent bone involvement. This fact has been confirmed in the literature as well as in clinical studies by many authors (Zappler

1948, Bruckner 1956, Massler 1958). The incidence of periodontitis in normal children is rare. Cohen and Goldman (1962), and Butler (1969) stated few cases of periodontitis. No further documentation of periodontitis in childhood was found except observations that its occurrence in children is infrequent.

The study of periodontal disease in children and adolescents is broad and includes all aspects of periodontal involvement: gingivitis, periodontosis, gingivostomatitis and gingival hyperplasia, (each may appear individually, as part of the syndrome, or concomitant with other general and systemic findings). This research project is limited to the study of CF in normal healthy gingiva and to the gingivitis provoked by poor oral hygiene, local factors, or puberty influences.

The techniques for measuring the CF are multiple, many problems related to the method of collection became obvious with the investigations of that fluid. Micro-capillary tubes have been used to collect the fluid determining directly the volume (Krasse and Egelberg 1962, Shillitoe and Lehner 1972). Twisted thread has been inserted into the crevice around the tooth and the difference in weight would give an idea of the volume (Weinstein 1967). These two methods are not common nor practical due to the length of time for collection and the ensuing inaccuracy. The most commonly used procedure for collecting the fluid and the most practical clinically has been the

placement of filter paper strips into or at the entrance of the crevice for a fixed period of time. The amount of fluid can be determined either by staining or viewing under a microscope, or by electronically determining the amount of fluid using a CF meter (Periotron, Harco Electronics, Winnipeg, Canada).

Sampling the CF by means of absorbing paper strips has been a matter of controversy between the investigators. The strips were used in two different ways: for the "intracrevicular" method, the end was gently inserted in the crevice; for the "extracrevicular" method, the strips were adapted on the vestibular surfaces of the teeth.

The "intracrevicular" method. This method is the most widely used. The filter paper is introduced in the gingival crevice until a retention or resistance would tend to prevent further insertion. Different techniques have been illustrated by Loe and Holm Pederson (1965), Brill (1958, 1962), and Mann (1963). The evaluation of the amount of fluids collected has been of great interest to evaluate the state of the gingiva which is directly related to the amount of fluid. A staining method has been proposed by Brill (1962) and Orban and Stallard (1969). A weighing technique has been used by Valazza (1972) where strips marked previously were inserted at one millimeter depth and weighed before and after insertion.

The "extracrevicular" method. Brilland Krasse (1958) used it on dogs, Loe and Holm Pederson (1965) widely used it in human sampling. The strips were made to fit closely to the buccal surface of the tooth, gingival margin and the attached gingiva.

Contamination by saliva may be avoided by different techniques. Mann (1963) described one of them proposing to place on both sides of the strip other strips to avoid contamination. A cotton roll in the vestibule with a stream of air and then inserting a strip to dry the sulcus from saliva contamination is another method.

CHAPTER III

MATERIALS AND METHODS

I. MATERIALS

The Patients

The patients which participated in this study were selected randomly from those treated at the Dental Clinic. Sixty children, in the range of seven to thirteen years of age were divided into two (2) groups of thirty (30) each, those with normal gingiva and those with inflammed simple gingivitis. The criteria of classification under normal and inflammed are the same as described earlier in the literature review. The patients with normal gingival tissue are those manifesting a light pink gingiva. This gingiva shows a smooth, soft, shiny surface with thick and rounded margins. The patients with inflammed gingival tissue are those with a mild inflammation of the papillae and marginal tissues, or a marginal gingival enlargement. That latter group represented poor oral hygiene, puberty gingivitis or other local factors provoking the inflammation. All the patients selected were healthy Caucasian children without known systemic or general disturbances. No sub-groups were observed and no difference in sex was taken into

consideration. No particular instructions were given to the patient. The patients were selected on the basis of clinical observation and evaluation and classified in one of the two (2) groups. The patient was introduced to the equipment and the crevicular fluid measurement was taken first utilizing the intracrevicular method; followed by the saliva test. In patients with normal gingiva, any of the six anterior teeth were selected for the test. However, when inflammed tissue was present, the tooth with the most inflammed marginal area was selected. After both tests were accomplished, a clinical oral examination and patient history was recorded. Any history of pain, gingival bleeding, poor oral hygiene, disturbed puberty or other medical background was taken into consideration for the classification of the patient. An important consideration was that the teeth selected had no caries, restorations, and were completely erupted without a remarkable plaque formation.

The PERIOTRON*

The Periotron is a gingival crevicular fluid flow meter. The meter contains two jaws that function like the plates of an electrical condenser. When a dry strip is inserted, the capacitance is at its maximum but with the appropriate electronic circuits registering zero on the viewer. Insertion of a wet strip provokes a reduction in

*PERIOTRON (Harco Electronics, Winnipeg, Canada).

the capacitance and results in a rise of the reading. This rise is directly proportional to the wet area, specifically to the volume of fluid collected. The jaws have an additional role of considerably reducing sample evaporation which is extremely important with minute samples. The manufacturer produces the filter strips which are sterile and homogenous. Standardized strips of the same length, width and depth, were marked by a line for insertion between the jaws of the Periotron in order to standardize the sampling. Because of the electronic device, a three second insertion would give an indication of the fluid flow with very little tissue irritation.

The GINDEX^{TM}*

This procedure was done to determine whether or not there is a parallel between the CF and the saliva in both categories of patients. This material uses a shade range, tube standardized by the Janar Co. containing 1/10 cc of 4% solution of O-Tolidine-Dihydrochloryde, and an activator (3% of hydrogen peroxide H_2O_2). Two drops of saliva added to two drops of activator in a tube were mixed. About ten minutes later the color of the solution in the tube was compared to the shade scale (the shades are numerically marked). A study by the manufacturer determined that the normal range of a healthy gingiva is between 40 and 60. The color becomes darker with the amount of

*GINDEX^{TM} (Janar Company, Grand Rapids, Michigan).

neutrophilic leukocytes present. The discovery of CF flow starts before clinical structural changes.

II. METHODS

The method of collecting CF used in this research was the intracrevicular method. Fluid is collected from the intracrevicular sulcus twice at five minute intervals. The region examined was isolated with cotton rolls and dried with air. The following procedures were utilized on all patients:

The patient was examined visually to determine whether he should be classified under: normal gingiva or inflammed gingiva. After the area was isolated and dried, a dry filter strip was placed in the crevice for three (3) seconds to empty the crevicular pool and removed. Twenty-seven seconds later a fresh filter strip was placed in the sulcus for three (3) seconds and the strip was immediately placed in the Periotron. The highest number was recorded and the procedure repeated after five minutes.

The patient was instructed to swish saliva between his teeth and expectorate into a vial to accumulate a volume of 1 cc.

A saliva test was determined using the Gindex by adding two drops of saliva and two drops of activator to a tube containing a predosed amount of reactor.

CHAPTER IV

EXPERIMENTAL RESULTS

I. EXPERIMENTAL DATA

Using the Periotron

Comparison of samples within a group. The CF measurement was monitored for the two groups. The child's CF was computed twice at five minute intervals:

1. The mean value of the initial measurement of the CF was: 6.43 for the normal gingiva, 16.8 for the inflammed gingiva.
2. After five minutes, a second measurement was done and the mean value was: 7.33 for the normal gingiva, 17.93 for the inflammed gingiva. (The measurements are given in Table III.)

A comparison of score values was examined and a t-Test was computed for CF difference which was $t = 1.867$ for the normal gingiva and $t = 2.256$ for the inflammed gingiva. (The values are given in Table IV.) The CF measurement change from the initial to the

second measurement did not show a statistically significant increase at P = .05 for the children with normal gingiva. (t = 1.867.)

The CF increase in the group of children with inflammed gingiva was statistically significant at P = .05. (t = 2.256.)

Comparison of samples between groups. The means of both measurements on each child in the first group were compared to those of the second group. The values are given in Tables I and II. Means, standard deviations and variances for the CF in both groups are given in Table VI.

A t-Test was computed for normal and inflammed CF values (Table VII). The CF scores, using the Periotron, showed a statistically significant increase between children with normal and inflammed gingiva at P = .05. (t = -9.504.)

Using the Gindex

The same experimental study was duplicated using the Gindex with only one sample being taken for each child. The Gindex score value for both groups is given in Tables I and II. A mean, standard deviation, and variance has been computed and the values are given in Table VI.

A t-Test was computed for the scores of both Gindex values (Table VII). The Gindex score change between the children with

normal gingiva and those with inflammed gingiva showed a statistically significant increase at $P = .05$. ($t = -5.144$.)

CHAPTER V

DISCUSSION

The results of this study show that there is an increase in CF flow in children with inflammed gingiva as compared to children with normal gingiva. It is important to consider the flow of fluid during the collection. Variations of flow would greatly affect any quantitative observations of gingival fluid. The t-Test showed that the comparison of the two collections with the Periotron of normal gingival CF is not statistically significant. On the other hand, the increase which occurs in the children with inflammed gingiva may be mainly due to the sampling method, the deep intracrevicular method being irritative in the children with an inflammed tissue. However, the comparison of the two groups using the Periotron is the consideration of this study. The results showed that the difference observed between the two categories of children is similar to that of the adults. The present study confirms the findings of the research done previously of children.

The use of the Gindex in this study was a complimentary test in order to find whether that relation remains the same using another method.

In this study, a comparison of two groups was studied to relate a normal gingiva to an inflammed gingiva. Other methods are still needed to add more scientific information on gingival inflammation in children. The utilization of homogenous groups and sub-groups might add further information to the present study.

Clinically, gingivae may appear normal, but may present sub-clinical inflammation. The prevention of further gingival involvement should be detected at this stage to avoid future periodontal disease (which has its origin in childhood).

CHAPTER VI

SUMMARY AND CONCLUSION

I. SUMMARY

Sixty children were selected randomly between the ages of seven and thirteen. They were divided into two groups of thirty each. One group of children with normal gingiva and the second with inflammed gingiva. They were classified into a group using the clinical appearance of the gingiva in the maxillary anterior segment. An intracrevicular fluid measurement using the Periotron was taken twice on each child at five minute intervals followed by a saliva test using the Gindex method to complement the findings.

The results showed a difference of CF values for the two groups of children. The comparison of the two measurements at five minute intervals on the same child did not show any difference for those with normal gingiva. However, children with inflammed gingiva showed an increase in CF flow. The use of a saliva test collaborated the findings of both categories of children.

The results of this study show similarity to the results previously found in adults. The treatment and prognosis of gingival

inflammation are not the same, but it is clear from the literature that early detection is followed by successful treatment.

II. CONCLUSIONS

1. There was no statistically significant difference between the two samples of CF flow at five minute intervals in the group of children with normal gingiva.

2. There was a statistically significant increase between the two samples of CF flow at five minute intervals in the group of children with inflammed gingiva.

3. There was a high statistically significant difference between both categories of children. The CF flow is significantly increased in the group of children with inflammed gingiva.

4. There was a similarity in results, using a saliva test. With the Gindex, there was a high statistically significant difference between both categories of children.

BIBLIOGRAPHY

BIBLIOGRAPHY

Ash, M. M., B. N. Gitlin and W. A. Smith. Correlation between plaque and gingivitis, J. Periodont., 35:248, 1964.

Baer, P. N., N. C. Brown and J. E. Hammer. Hypophosphatasis, Periodontics, 2:209, 1964.

Baer, P. N., P. J. Coccaro, M. J. Baer and Kilham. Craniofacial manifestations of virus induced mongolism in the hamster and Down's syndrome in man, Am. J. Orth., 60:221, 1971.

Baer, P. N. and S. D. Benjamin. Periodontal Disease in Children and Adolescents. Textbook, 1974.

Benjamin, S. D. and P. N. Baer. Familial patterns of advanced alveolar bone loss in adolescence (periodontosis), Periodontics, 5:82-88, 1967.

Borden, S. M., L. M. Golub and I. Kleinberg. An intracrevicular technique for monitoring gingival crevice fluid, J. Dent. Res., 53, 1974.

Borden, C., L. M. Golub and I. Kleinberg. The effects of age and sex on urea concentration in gingival crevice fluid in humans, (unpublished), 1975.

Brill, N. and B. Krasse. The passage of tissue fluid into the clinically healthy gingival pocket, Acta Odont Scand, 16:233-245, 1958.

Brill, N. and H. Bjorn. Passage of tissue fluid into human gingival pockets, Acta Odont Scand, 17:11-21, 1959.

Brill, N. Influence of capillary permeability on flow of tissue fluid into gingival pockets, Acta Odont Scand, 17:23-33, 1959.

Brill, N. Gingival conditions related to flow of tissue fluid into gingival pockets, Acta Odont Scand, 18:421, 1960.

Brill, N. and R. Bronnestam. Immunoelectrophoretic study of tissue fluid from gingival pockets, Acta Odont Scand, 18:95, 1960.

Brill, N. The gingival pocket fluid, Acta Odont Scand, 20 (Supp. 32), 1962.

Bruckner, M. Gingivitis and Vincent's infection in children, J. Dent. Child, 23:116, 1956.

Butler, J. H. A familial pattern of juvenile periodontitis (periodontosis), J. Periodont, 40:115, 1969.

Cimasoni, G. The crevicular fluid, Monographs in Oral Science, Vol. No. 3, 1974.

Cohen, M. M. The effect of large doses of ascorbic acid on gingival tissue at puberty, 5, Dent Res., 34:750, 1955.

Cohen, M. M. The gingiva at puberty, J. Dent Res., 35:679, 1955.

Cohen, D. W. and H. M. Goldman. Periodontal disease in children, PDM, I, July, 1962.

Egelberg, J. Gingival exudate measurements for evaluation of inflammatory changes of the gingiva, Odont Rev., 15:381-398, 1964.

Egelberg, J. Permeability of the dento-gingival blood vessels, J. Periodont. Res., I:180-191, 276-286, 287-296, 1966.

Emslie, R. D. Cancrum Oris, Dent Pract Dent Rec., 13:481, 1963.

Fitzgerald, R. J., H. V. Jordan and H. R. Stanley. Experimental caries and gingival pathologic changes in the gnotobiotoc rat, J Dent Res., 39:923, 1960.

Frostell, G. Studies on the ammonia production and the ureolytic activity of dental plaque material, Acta Odont Scand, 18:29-65, 1960.

Fullmer, H. M., P. N. Baer, E. J. Driscoll and P. M. Lightbody. Collagenase and gingival diseases, Proc. of the first Pan-Pacific Cong, Dent Res., 161-171, 1970.

Gibbons, R. J. and J. B. MacDonald. Degradation of collagenous substarates by bacteroides melaninogenicus, J. Bacteriol, 81:614-621, 1961.

Golub, L. M., S. M. Borden and I. Kleinberg. Urea content of gingival crevicular fluid and its relation to periodontal disease in humans, J. Periodont. Res., 6:243-251, 1971.

Golub, L. M., J. E. Stakiw and D. L. Singer. Collagenolytic activity of human gingival crevice fluid, J. Dent. Res., 53:1501, 1974.

Greene, J. C. Periodontal disease in India, J. Dent. Res., 39:302, 1960.

Hirsch, J. Periodontal disease in children: Study of etiological factors in childhood, D. Student's Mag., 34:24, 1955.

Johnson, N. P. and M. A. Yound. Periodontal disease in mongols, I, Periodont., 34:41, 1963.

Jordan, H. V., R. J. Fitzgerald and H. R. Stanley. Plaque formation and periodontal pathology in gnotobiotic rats infected with oral actinomycete, Am. I Pathol., 47:1157, 1965.

Kaslick, R. S., A. J. Chasens, D. Weinstein, R. Waldman, J. Pluhar and R. Lazzara. Quantitative analysis of sodium, potassium and calcium in gingival fluid from gingiva with varying degrees of inflammation, J. Periodont., 41:93-98, 1970.

Kleinberg, I. Regulation of the acid-base metabolism of the dento gingival plaque and its relation to dental caries and periodontal disease, Int. Dent. Journal, 20:451, 1970.

Kleinberg, I. The role of dental plaque in caries and inflammatory periodontal disease, J. Canad. Dent. Assoc., 40:56-66, 1974.

Krasse, B. and J. Egelbert. The relative proportion of sodium, potassium and calcium in gingival pocket fluid, Acta Odont Scand., 20:143-152, 1962.

Loe, H. and P. Holm-Pederson. Abscence and presence of fluid from normal and inflammed gingiva, Periodontics, 3:171-177, 1965.

Loe, H., E. Theilade and Jensen S. Borglum. Experimental gingivitis in man, J. Periodont., 36:177-187, 1965.

McCall, J. O. Gingival and periodontal disease in children, J. Periodont., 9:7, 1938.

McCombie, F. and D. Stothard. Relationships between gingivitis and other dental conditions, J. Canad. Dent. Assoc., 30:506, 1964.

MacDonald, R. E. and Aranjo. The gingival crevice microbiotic of preschool children, J. Periodont., 35, 1964.

MacDonald, R. E. Gingivitis and periodontal disease in children, Dentistry for the Child and Adolescent, 223, 1974.

Mann, W. V. The correlation of gingivitis, pocket depth and exudate from the gingival crevice, J. Periodont., 34:379-387, 1963.

Mann, W. V. and H. Stoffer. The identification of protein components in fluid from gingival pockets, Periodontics, 2:263, 1964.

Massler, M., I. Schour and B. Chopra. Occurrence of gingivitis in suburban Chicago School Children, J. Periodont., 21:146, 1950.

Massler, M., A. Cohen and I. Schour. Epidemiology of gingivitis in children, J. Am. Dent. Assoc., 45:319, 1952.

Massler, M. Periodontal disease in children, Int. Dent. Journal, 3:323, 1958.

Oliver, R. C., P. Holm-Pederson and H. Loe. The correlation between clinical scoring, exudate measurements and microscopic evaluation of inflammation in the gingiva, J. Periodont. Res., 40:201-209, 1969.

Orban, J. E. and R. E. Stallard. Gingival crevicular fluid: A reliable predictor of gingival health? J. Periodont., 40:231-235, 1969.

Ramfjord, S. P. Periodontal Status of Boys in Bombay, J. Periodont., 32:237, 1961.

Ramfjord, S. P. Epidemiological studies of periodontal diseases, Parondont Acad. Rev., 2:109, 1968.

Raphael, A. L., P. N. Baer and W. B. Lee. Hyperkeratosis of gingival and plantar surfaces, Periodontics, 6:118, 1968.

Robinson, H. G. Periodontitis and periodontosis in children and young adolescents, J. Am. Dent. Assoc., 43:709, 1951.

Rudin, H. J., H. F. Overdick and K. H. Rateitschak. Correlation between sulcus fluid rate and clinical and histological inflammation of the marginal gingiva, Helv. Odont. Act., 14:21-26, 1970.

Salind, A., H. J. Oshrain and I. D. Mandel. Observations on gingival pocket fluid, Periodontics, I:196, 1963.

Shillitoe, E. J. and T. Lehmer. Immunoglobulins and complement in crevicular fluid, serum and saliva in man, Arch. Oral Viol., 17:241-247, 1972.

Singer, D. L. and I. Kleinberg. Ammonia and urea content of maxillary and mandibular incisor plaques, (unpublished), 1975.

Smith, P. and K. A. Rosenzweig. Seven cases of Papillon Le febre syndrome, Periodontics, 5:42-46, 1967.

Smith, L. B., J. T. Rule and S. Rosen. Evaluation of collagenase activity in the crevicular fluid of children, J. Dent. Res., 53:150, 1974.

Theilade, E., W. H. Wright, S. J. Borglum and H. Loe. Experimental gingivitis in man, J. Periodont Res., I:1-13, 1966.

Turesky, S. Histologic and histochemical Observations regarding early calculus formation in children and adults, J. Periodont, 32:7, 1961.

Valazza, A., J. Matter, A. Ogilvie and G. Cimasoni. Gingival fluid, gingival inflammation, pocket depth and bone loss, Odontostomat of Switzerland, 82:824-832, 1972.

Weinstein, E. and I. Mandel. The fluid of the gingival sulcus, Periodontics, 2:147-153, 1964.

Weinstein, E., I. D. Mandel, A. Salkind, H. I. Oshrain and C. D. Pappas. Studies of gingival fluid, Periodontics, 5:161-166, 1967.

Zappler, S. E. Periodontal disease in children, J. Am. Dent. Assoc., 37:333, 1948.

APPENDIX

APPENDIX A

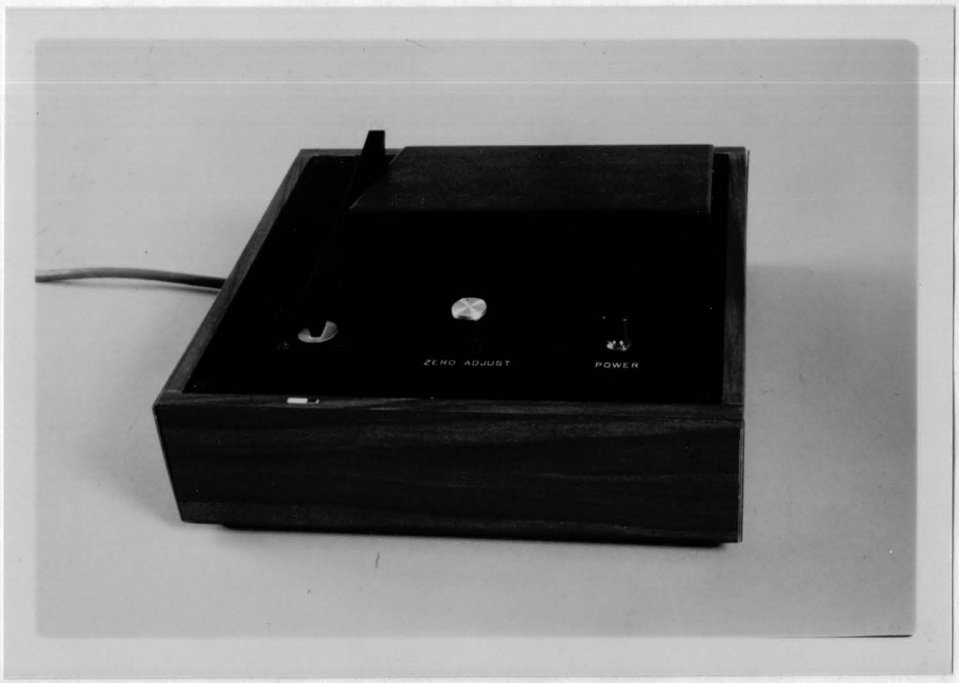

PLATE 1

PERIOTRON

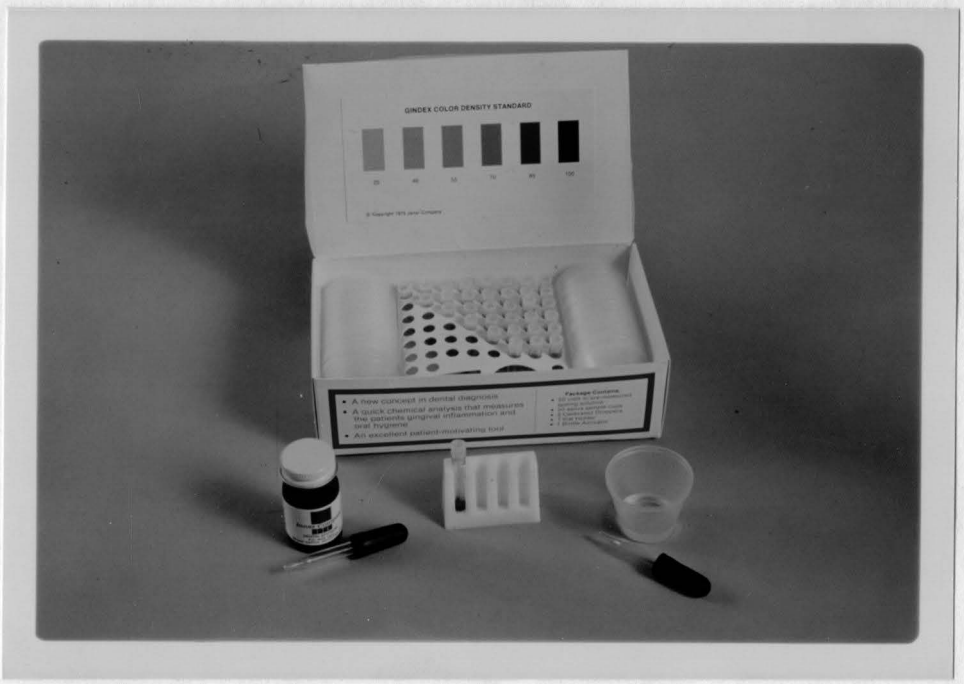

PLATE 2

GINDEX™

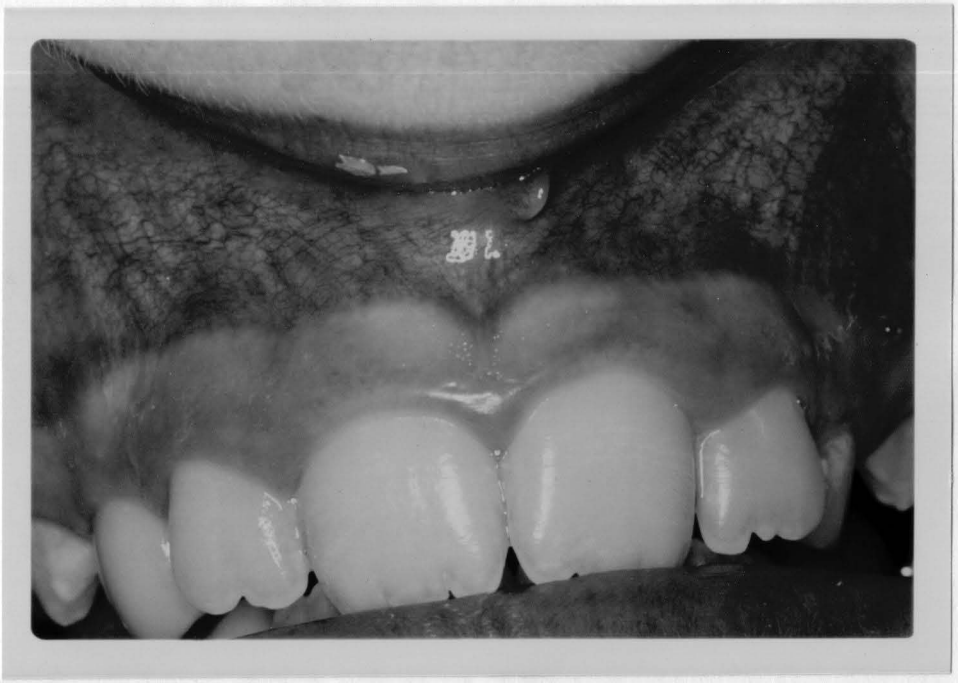

PLATE 3

NORMAL GINGIVA

PLATE 4

INFLAMMED GINGIVA

APPENDIX B

TABLE I

METER SCORES - NORMAL GINGIVA

Patient	Sample #1	Sample #2	Mean of CF with Periotron	Value of Gindex
1	5	7	6	70
2	2	8	5	85
3	6	4	5	40
4	6	6	6	55
5	9	11	10	70
6	3	4	3.5	25
7	8	8	8	50
8	3	3	3	25
9	7	13	10	80
10	11	13	12	60
11	10	8	9	75
12	6	9	7.5	55
13	3	3	3	40
14	7	9	8	65
15	2	3	2.5	55
16	8	6	7	75
17	7	13	10	75
18	5	2	3.5	65
19	11	9	10	85
20	9	10	9.5	75
21	6	6	6	55
22	4	8	6	100
23	3	3	3	60
24	6	6	6	65
25	10	8	9	55
26	6	6	6	50
27	7	13	10	70
28	10	7	8.5	60
29	8	9	8.5	40
30	5	5	5	95

APPENDIX C

TABLE II

METER SCORES - INFLAMMED GINGIVA

Patient	Sample #1	Sample #2	Mean of CF with Periotron	Value of Gindex
1	14	18	16	90
2	18	18	18	75
3	15	17	16	85
4	15	15	15	85
5	16	14	15	70
6	18	18	18	70
7	23	21	22	95
8	26	26	26	100
9	22	22	22	100
10	15	15	15	80
11	15	15	15	80
12	26	26	26	85
13	27	32	29.5	95
14	12	15	13.5	80
15	12	22	17	80
16	22	21	21.5	75
17	19	21	20	75
18	14	16	15	90
19	9	13	11	70
20	15	15	15	75
21	35	31	33	80
22	14	16	15	85
23	10	14	12	75
24	15	17	16	75
25	12	16	14	85
26	13	13	13	70
27	12	11	11.5	80
28	12	11	11.5	75
29	14	17	15.5	85
30	14	12	13	75

APPENDIX D

TABLE III

COMPARISON OF SCORE VALUES AT FIVE MINUTE INTERVALS WITH THE PERIOTRON

Normal (A-B)				Inflammed (A-B)			
A	B	D	D^2	A	B	D	D^2
7	7	2	4	18	14	4	16
8	2	6	36	18	18	0	0
4	6	-2	4	17	15	2	4
6	6	0	0	15	15	0	0
11	9	2	4	14	16	-2	4
4	3	1	1	18	18	0	0
8	8	0	0	21	23	-2	4
3	3	0	0	26	26	0	0
13	7	6	36	22	22	0	0
13	11	2	4	15	15	0	0
8	10	-2	4	15	15	0	0
9	6	3	9	26	26	0	0
3	3	0	0	32	27	5	25
9	7	2	4	15	12	3	9
3	2	1	1	22	12	10	100
6	8	-2	4	21	22	-1	1
13	7	6	36	21	19	2	4
2	5	-3	9	16	14	2	4
9	11	-2	4	13	9	4	16
10	9	1	1	15	15	0	0
6	6	0	0	31	35	-4	16
8	4	4	16	16	14	2	4
3	3	0	0	14	10	4	16
6	6	0	0	17	15	2	4
8	10	-2	4	16	12	4	16
6	6	0	0	13	13	0	0
13	7	6	36	11	12	-1	1
7	10	-3	9	11	12	-1	1
9	8	1	1	17	14	3	9
5	5	0	0	12	14	-2	4

APPENDIX E

TABLE IV

STUDENT t-TEST

Normal Gingiva	Inflammed Gingiva
$\Sigma D_N = 27$	$\Sigma D_I = 34$
$\bar{D}_N = 0.9$	$\bar{D}_I = 1.133$
$S_D = 2.643$	$S_D = 2.750$
$T = 1.867$	$T = 2.256$

APPENDIX F

TABLE V

MEANS OF SCORES

Normal Gingiva				Inflammed Gingiva			
Periotron		Gindex		Periotron		Gindex	
X_N	X^2_N	Y_N	Y^2_N	X_I	X^2_I	Y_I	Y^2_I
6	36	70	4900	16	256	90	8100
5	25	85	7225	18	324	75	5625
5	25	40	1600	16	256	85	7225
6	36	55	3025	15	225	85	7225
10	100	70	4900	15	225	70	4900
3.5	12.25	25	625	18	324	70	4900
8	64	50	2500	22	484	95	9025
3	9	25	625	26	676	100	10000
10	100	80	6400	22	484	100	10000
12	144	60	3600	15	225	80	6400
9	81	75	5625	15	225	80	6400
7.5	56.25	55	3025	26	676	85	7225
3	9	40	1600	29.5	870.25	95	9025
8	64	65	4225	13.5	182.25	80	6400
2.5	6.25	55	3025	17	289	80	6400
7	49	75	5625	21.5	462.25	75	5625
10	100	75	5625	20	400	75	5625
3.5	12.25	65	4225	15	225	90	8100
10	100	85	7225	11	121	70	4900
9.5	90.25	75	5625	15	225	75	5625
6	36	55	3025	33	1089	80	6400
6	36	100	10000	15	225	85	7225
3	9	60	3600	12	144	75	5625
6	36	65	4225	16	256	75	5625
9	81	55	3025	14	196	85	7225
6	36	50	2500	13	169	70	4900
10	100	70	4900	11.5	132.25	80	6400
8.5	72.25	60	3600	11.5	132.25	75	5625
8.5	72.25	40	1600	15.5	240.25	85	7225
5	25	95	9025	13	169	75	5625

APPENDIX G

TABLE VI

STANDARD DEVIATIONS AND VARIANCES

$\Sigma X_N = 206.5$	$\Sigma Y_N = 1875$	$\Sigma X_I = 521$	$\Sigma Y_I = 2440$
$\overline{X}_N = 6.883$	$\overline{Y}_N = 62.5$	$\overline{X}_I = 17.366$	$\overline{Y}_I = 81.333$

Value with Periotron

Normal	Inflammed
$S_{X_N} = 2.634$	$S_{X_I} = 5.4439$

Value with Gindex

Normal	Inflammed
$S_{Y_N} = 18.135$	$S_{Y_I} = 8.6036$

APPENDIX H

TABLE VII

t-TEST BETWEEN NORMAL AND INFLAMMED GINGIVA WITH THE PERIOTRON AND THE GINDEX

Periotron	Gindex
Normal - Inflammed Gingiva	Normal - Inflammed Gingiva
S_p = 4.276	S_p = 14.193
T = -9.504	T = -5.144

APPENDIX I

TABLE VIII

CREVICULAR FLUID COMPOSITION*

Cellular Elements

1. Epithelial Cells
2. Leukocytes
3. Bacteria

Electrolytes

1. Sodium
2. Potassium
3. Other ions mainly Calcium, Chloride, Magnesium

Organic Compounds

1. Carbohydrates
2. Proteins (γ , β , α_2 , α_1 , globulins, albumins

Metabolic and Bacterial Products

1. Lactic Acid
2. Urea
3. Hydroxyproline
4. Endotoxins
5. Cytotoxic Substances
6. Antibacterial Factors

Enzymes

1. Acid Phophatase
2. Alkaline Phosphatase
3. β -Glucuronidase
4. Lysozyme
5. Cathepsin D.
6. Proteases
7. Lactic Dehydrogenase

*Cimasoni, 1974

TABLE IX

FLORA IN GINGIVAL CREVICE*

$Gram^{+}_{+}$ facultative rods	29.7%
$Gram^{+}_{+}$ facultative cocci	21.9%
$Gram^{-}_{+}$ anaerobic cocci	16.3%
$Gram^{+}$ anaerobic rods	16.3%
$Gram^{-}$ anaerobic rods	8.8%
$Gram^{+}$ anaerobic cocci	3.5%
$Gram^{-}$ facultative rods	1.8%
$Gram^{-}$ facultative cocci	1.7%

*MacDonald-Azanjo (1964)

APPROVAL SHEET

This thesis, submitted by Rafik Ferdinand Hakim, has been approved by the following Committee:

Dr. Wayne E. Milos, Assistant Professor, Director of Pedodontic Graduate Students, Department of Pedodontics, Loyola University School of Dentistry

Dr. Eugene R. Grandel, Professor and Chairman, Department of Pedodontics, Loyola University School of Dentistry

Dr. Patrick D. Toto, Professor and Chairman, Department of Oral Pathology, Loyola University School of Dentistry

The final copies have been examined by the Director of the thesis, and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

5/5/77
Date

Dr. Wayne E. Milos
Signature of the Director